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DEPARTMENT OF THE ARMY
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SOMETHING ABOUT THE SYMBIONTS OF MOSQUITOES

Following is the translation of an article by Antonio Verdarol Arnal published in Bol. soc. espan. hist. nat. Madrid. Vol. 41 :209-213. 1943. Translation by Bernice MacDonald.

The descriptions of the digestive apparatus of the Culicidos of Le Danteq, Nuttall and Shipley and Sagaudinn are classic. We will deal here withthe most interesting of these.

After a muscular pharynx is a thin esophagus which dilates at the end to form a still quitinose proventricle. The back of this forms a valve with a sfincter which separates it from the middle intestine. On the walls of the proventricle there are microscopic quitinos projections which stem from each cell separately. They strongly resemble those described in Calliphora by Berlesse but they are longer and more slender. Here the esophagus forms three evaginations, two dorsal and one ventral. This one is a large sac which extends underneath the digestive tube as far as to fill a great part of the abdomen attaining its greatest developemnt in the Anopheles. Nuttall and Shipley consider the three to be food reservoirs. Other authors noticing that similar sacs in other diptera occassionally contain food think it very likely that such diverticula serve as storehouses. Schaudinn calls the ventral

diverticulum a sucking stomach (Saugmagen), which will fill up with blood during suction and which would empty itself immediately into the middle intestine. Nuttall and Shipley give it the same meaning, but to Berlesse it is a simple air sac, destined to maintain the shape of the body when the digestive tube is empty and to leave room for it when it distends upon filling up with blood. These two functions do not seem contradictory as we have observed that the mentioned diverticulum appears as a virtual cavity in fasting mosquitoes, while it appears muscular when the animal sucks blood.

During the fasting period the lower part of the proventricle is folded inside the part close to the middle intestine, which dilates, giving it a pear-shaped appearance. Some authors mistakenly call this entire area cardias. The name stomach, confirmed by comparative anatomy, would better suit it.

With the ingestion of blood the proventricle disinvaginates and dilates to the shape of a winebag, its cells separated by a gelatinous substance like quitina (?). This gelatine will surround the mass of blood during its passage to the middle intestine and will form a kind of peritrophic membrane although it is of a different origin than that of the rest of the insects.

The wall of the middle intestine, of endothermic

origin is very monotonous. It is made up (a in Pic. VI, fig. B) of prismatic cells with a thick nucleus which in in apical part have a protective sheet stretched vertically, called rhabdorium.(b). For some authors the rabdorium whould also have an absorbent function. These are the cells which separate the digestive enzymes.

In the middle intestine the digestion and absoption of the food takes place, we dont know if through the same cells as Boissezon says these are separated into absorbent and secreting cells to better fulfill these functions. The secreting rhythm is not the same during fast (secretion of hunger) as during contact with food. We, along with Boissezon, also differentiate two kinds of cells for these two secretions.

In the posterior area the so called piloric valve separates the middle intestine from the posteriro intestine which again becomes quitinos. on the outside the middle intestine is surrounded by a strong muscular tunic and has a profusion of tracheas.

Forty years ago Schaudinn pointed out the exsistence of some chainlike fungi in the ventral sac of the Anopheles which until then was supposed to be only an air sac. The function of these fungi

[illegible] presumed to [illegible] enzymes and at the same [illegible] gas [illegible] bubbles. Schaudinn supposed that these enzymes are the ones which produce in inflamation of the bite. He saw that the gas porduced was CO_2 as it clouded barium water. These funguses which he presumed to be [illegible] were of great importance to the insect. These [illegible] exsist in the eggs. For Schaudinn they would be hereditary residing in internal tissues from which they would pass to the eggs.

More recently, Hertig and Wolbach have found them again in Culex. In the testicles and ovaries of the males and females there are slender little intercellular canes similar to gramnegative Rickettsias. Some of other species have been identified with Schaudinn's fructiferous forms but in others the same result was not obtained.

Therefore, it is not a case of casual infection. These studies prove that they are real specific symbiontes which in all probability intervene in the nutrition of the host.

I have used for my first investigations three species of very abundant hematophages Culicidos: Anopheles, Cubex and Aedes (Stegomia), hunting them by the simple means of placing on them, when they are poised, a large diameter test tuble. By introduring into the same tube which served to capture

them a strip of paper where they could poise, they can be kept alive for several days.

I effected the fixing "in toto" in formaldehyde deacidified using chalk in the ratio of 10 to 100; I have made the cuts before inclusion in celodene. For dyeing I have used various techniques: especially the first variant of Rio-Hortega to the tonoargentic method of Achucarro, and Rio-Hortega's silver carbonate method.

Treatment of the whole insect offers the grave inconvenience of defective inclusion. Even in the most favorable cases there always remains a limit marked by the quitine. For this reason the body siluette of the body breaks away as soon as rather fine cuts -10 u- are tried. These are necessay for the histophysiologic studies as the cells of the mosquito are very small.

I am now trying another procedure, not lacking in difficulties either. It consists in a previous dissection of the organs (digestive tube, salivary etc.) before fixinf and including them. But this can not be done when the digestive tube is full of blood, which is the most interesting case.

The mosquitoes which have just sucked blood have ~~at first sight the~~ a dilated abdomen ~~dilated and full of it. It~~ which can be observed in a sagittal cut ~~(Pic. VI~~

[illegible] the wall of the middle intestine is distended (a) and its cells have lost height. The intestinal content has zones more altered than others where the red blood cells have hemolyzed and some have broken, showing only membranes. These zones where hemolysis has started are distributed with preference to the back (c") and in a central mass (c-c') which advances from the cardias.

Observing in detail (pic. VI. fig. B) this zone of hemolysis, there are seen among the red blood cells (f), more or less altered, a profusion of bacillary bacteria, on occassion lumped together and also forming chains. The hematies situated in the vicinity of the groups of bacterias are hemolyzed, and broken, while leucocytes are always unaltered (g). This fact makes me suppose that the bacterias are there to cause hemolysis. The role of this preparatory step would limit it in digestion, as the real digestive action is the exclusive work of the digestive juices, separated by the cells of the middle intestine, whichwwould surely fo to the interior of the mass of blood through the peritrophic gelatine (e).

Boissezon, in his work onthe digestion of blood in Culex, says that hemolysis and later crystalization are produced by the action of the

digestive juices. But, he does not mention these bacteria at all whic he probably has not seen. As can be observed in pic. VI, fig. A. this accumulation of bacteria penetrates to the interior of the mass of compressed blood and it forges galeries and sponges it with the help of the gases released, aidinf in this way the digestive enzymes which have to act from the perifery.

If, as Nuttall and Shipley suppose, the blood absorbed passes pseviously through the esophagic diverticula, it seems very likely that it load it self with the bacteria contained in these. In this was the gelatinous ampule surrounds both and constitues a nutritious bin already prepared for easy digestion.

Considering the Saugmagen of Schaudinn as a bacteria reservoir, that is, of hemolitic enzymes, the proventricle and its annexed diverticula would be the real stomach where the food would be prepared beforing passing to the middle intestine.

We do not have enough elements to identify these bacteria. In Culex they are deplobacillus sometimes simple and more rarely forming chains (fig. I, a). In Anopheles they are rounded and always form short chains similar to yeasts (fig. I, b). It is necessary to identify them to use adequate bacteriologic methods and if possible obtain cultures.

It gives me great pelasure to make use of this

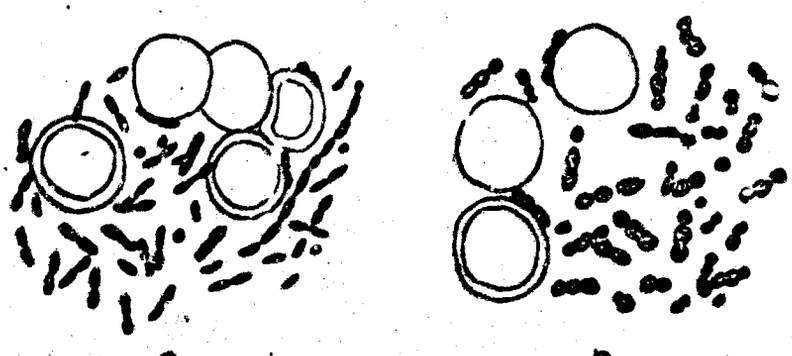

Fig. 1.—Simbiontes de *Culex* (a) y de *Anopheles* (b) con glóbulos rojos humanos. (Obj. Zeiss imm. hom. 90 K ; oc. 20 K.)

Fig. I Symbiontes of Culex (a) and of Anopheles (b) with human red blood cells.
(Obj. Zeiss inm. hom. 90X; oc. 20X.)

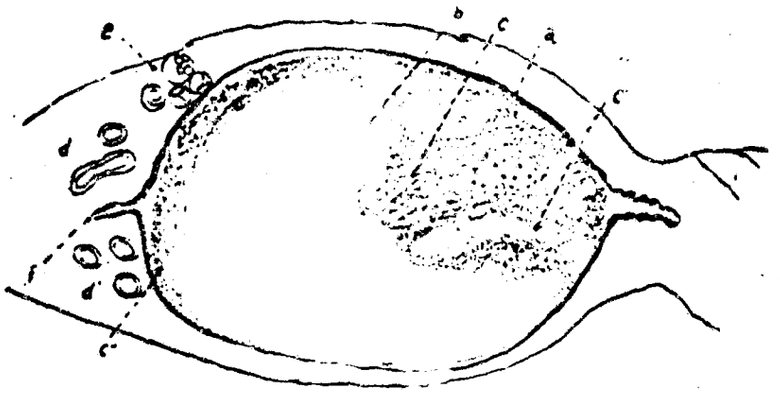

Fig. A.- Saritall cut of Culex 2 hours after sucking blood. Method: Rio-Ortega's silver carbonate: a, wall of the middle intestine, distended; b, mass of blood not hemolysized; c c' c", principal zones hemolysis with abundant bacteria; d d', Malpighi tubes; e, ovule; f, posterior intestine.

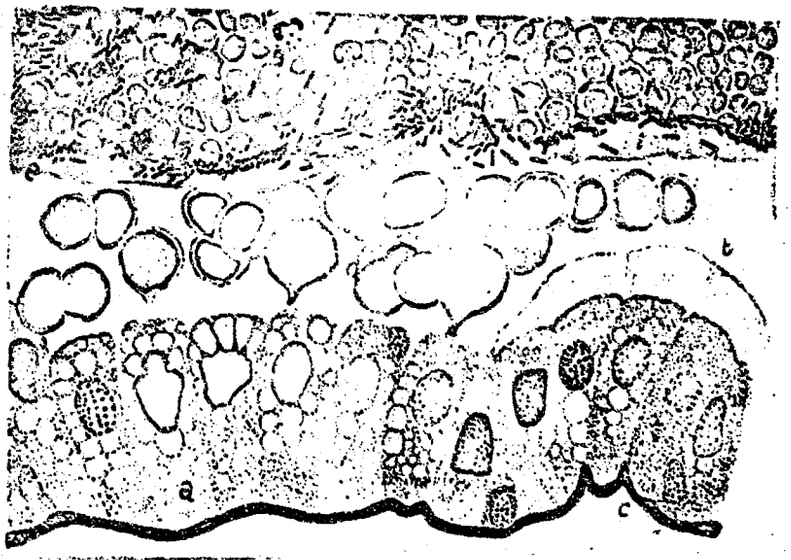

Fig. B. Details of the previous figure at c' level. a; wall of middle intestine; cells in different phases of secretion; b, rabdorium; 6, basal membrane of the epithelium; d, heavy drops of digestive juices; e, surrounding of gelatine with bacteria; f, hematites more or less hemolysed, with abundant compressed bacteria and in chains; g, unaltered leucocytes.

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